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Editorial

AMMONIA: MORE THAN A NEUROTOXIN?

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Ammonia is an inorganic nitrogen compound that is metabolized and produced in all tissues via a number of important biochemical reactions. However, a major source of ammonia generation occurs in the gut following protein digestion and amino acid deamination. The subsequent ammonia-rich portal blood is effectively removed by the urea cycle in a healthy liver, maintaining circulating concentrations of ammonia within 35–65 μM. When liver function is compromized, hyperammonaemia arises delivering toxic concentrations of ammonia to the brain, causing deleterious effects.

Ammonia is composed of a gaseous (NH_3) and ionic (NH_4^+) component, where the ratio of NH_3/NH_4^+ is a function of pH defined by the Henderson–Hasselbach equation. Under normal physiological conditions (pH 7.4) more than 98% of ammonia is present as NH_4^+. The ionic properties of NH_4^+ are unique in comparison to other weak bases, as NH_4^+ has a comparable ionic radius and diffusion coefficient as potassium (K^+). Therefore, in addition to ammonia diffusing into cells as a gas (NH_3), NH_4^+ can cross plasma membranes via K^+ transporters/channels.

Elevated levels of ammonia lead to new equilibriums across plasma membranes, which in turn elicit alterations in pH. In addition to high ammonia having an effect on cellular metabolism, an increase in NH_4^+ will specifically have an immediate impact on the membrane potential. These direct effects of increased ammonia will consequently trigger a cascade of pathophysiological events [1]. Although ammonia can disperse and enter all organs throughout the body, it is the brain that bears the brunt, as episodes of hyperammonaemia prompt neurological decline. Acquired hyperammonaemia, as observed during liver disease/failure, consequently leads to hepatic encephalopathy which includes an array of neuropsychiatric abnormalities from cognitive deficits to ataxia, stupor and coma. Inherited hyperammonaemia, as observed in children born with genetic defects in the urea cycle, causes neurological posturing, lethargia and seizures. In both cases, ammonia-lowering strategies remain a primary therapeutic target [2].

The study by Jia *et al*., published in Liver International [3] provides evidence that the toxic effects of ammonia are not restricted to the brain, and that elevated concentrations of blood ammonia cause hepatic dysfunction. Jia and colleagues demonstrated that increasing blood ammonia levels to concentrations between 120–180 μM for 4 weeks (attained through administration of ammonia by gavage twice a day to naïve rats) leads to alterations in liver biochemistry (including alanine/aspartate transaminases and total bilirubin) and detection of apoptotic cells in liver (quantified by number of TUNEL-positive cells). Histological analysis revealed no cell degeneration or regeneration, no cell necrosis and no inflammatory infiltration, however, oedematous hepatocytes were noted. The concept that hyperammonaemia, commonly arising under conditions when the liver's capacity to remove ammonia is reduced causes hepatic damage and hepatocyte apoptosis is paradoxical. For centuries, the liver has been defined as a vital organ for regulating and detoxifying ammonia. Whether ammonia can inadvertently affect the liver, it's primary source of detoxification, and hence perpetuate a vicious cycle is captivating and further studies are warranted to clarify this dispute.

The conclusions by Jia *et al*., stipulating hyperammonaemia impairs the liver leading to hepatic damage and hepatocyte apoptosis is intriguing, as to date there is little compelling evidence supporting this observation. In studies involving ammonia and different *in vivo* and *in vitro* model systems of the liver, hepatocyte cell death was rarely reported [4]. Furthermore, children with inborn errors of the urea cycle infrequently present with hepatocyte apoptosis. Conversely, mild hepatic fibrosis, glycogen accumulation and hepatocyte swelling have been found in these young patients, which are assumed to be related to age as the progression to these liver abnormalities is slow [5]. Moreover, these pathological findings are believed to be related to the type of urea cycle disorder (specific gene defect) and the accumulation of respective urea cycle intermediates. Further evidence describing ammonia does not impact on liver function, comes from

our group, where we recently demonstrated that by preventing the development of hyperammonaemia in bile-duct ligated-induced cirrhotic rats with AST-120 (carbon microspheres), the degree of liver damage was not alleviated [6]. In addition, Jover and colleagues elegantly demonstrated that inducing hyperammonaemia by feeding naïve rats with a high ammonia diet did not result in alterations in liver biochemistry [7]. Also, congenital portal-systemic shunting-induced hyperammonaemia does not result in liver damage [8, 9] and moreover rats with portacaval anastomosis (100% portal-systemic shunting) causing hyperammonaemia and encephalopathy, do not develop hepatic failure [10]. However, portal-systemic shunting does lead to reduction in liver mass and functional capacity [11, 12]. Nevertheless, this is believed to occur as a result of a decrease in liver perfusion (associated with degree of portal systemic shunting) [13] and not ammonia toxicity. Taken together, these studies strongly suggest that high ammonia does not impact on liver function and incur damage.

After revealing hepatocyte apoptosis befalls in this ammonia-toxicity rat model, it would have been of utmost value to probe for the detection of apoptotic cells in the brains of these same animals. This would have provided insights, in this model, on whether the brain and liver react similarly following the ammonia administrations. The underlying reason(s) why ammonia is particularly toxic to the brain, or why the brain is more susceptible to ammonia toxicity in comparison to other organs, remains incompletely understood. However, knowing the brain accounts for less than 2% of a person's weight but consumes 20% of the body's energy, these high energy requirements render the brain particularly susceptible to alterations in energy metabolism. It has been shown that neurotoxic concentrations of ammonia in the brain lead to alterations in the TCA cycle (affecting α-ketoglutarate dehydrogenase activity) [14] but neuronal apoptosis is seldomly reported.

In fact, historically, hepatic encephalopathy has always been considered to be a reversible metabolic disorder and therefore is expected to completely resolve following liver transplantation. However, even following the implantation of a new liver, persisting neurological complications remain a common problem affecting as many as 47% (8–47%) of liver transplant recipients [15, 16]. Consequently, these enduring neurological complications post-liver transplantation continue to weigh severely on the patients' quality of life, and also lead to longer stays in the hospital causing further financial burden on healthcare systems [17]. As a result, the reversibility of hepatic encephalopathy is questioned [18]. Given the success rate of liver transplantation is close to 90%, this surgery is no longer considered an experimental high-risk procedure, and thus the focus on patient survival has now shifted towards quality of outcome, including neurological status. These incidences of neurological impairment following liver transplantation underscore the need for careful neurological assessment in patients with liver disease/failure. It is understood that hyperammonaemia plays a primary role in hepatic encephalopathy and it has been demonstrated that under certain pathological conditions during liver disease, neuronal cell death ensues [19]. The precise role of ammonia and the implications of neuronal cell death in the pathogenesis of hepatic encephalopathy as well as in the persisting neurological complications following liver transplantation merits to be further explored.

In addition to impinging on the brain (and the liver as demonstrated by Jia *et al*.,), ammonia toxicity has also been shown to be implicated in respiratory diseases [20], to cause an upregulation of myostatin [21] and to trigger gastric epithelial cell death [22]. Understanding that increased ammonia affects metabolism and physiology in all cells, it is not astonishing that ammonia toxicity can impact on all organs. In fact, patients with liver disease regularly develop multi organ failure which leads to poor prognosis. Therefore, hyperammonaemia and the involvement of ammonia toxicity in the pathogenesis of extrahepatic organ dysfunction (including apoptotic cell death) remains poorly studied and warrants to be investigated.

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